

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
BEFORE THE BOARD OF PATENT APPEALS & INTERFERENCES**

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Group Art Unit: 1652

Applicant(s): HUANG et al.

Examiner: Hutson, Richard D.

Title: **METHOD OF INACTIVATING RIBONUCLEASES AT HIGH TEMPERATURE**

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**APPELLANTS' APPEAL BRIEF  
(37 CFR §41.37)**

**On Appeal from Art Unit 1652  
Examiner: Richard G. Hutson, Ph.D.**

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## REAL PARTY IN INTEREST

The real party in interest is Promega Corporation, which is the owner of the present application by virtue of the assignment recorded at Reel 16343/Frame 274.

## RELATED APPEALS AND INTERFERENCES

U.S. Application No. 10/403,395, currently on appeal, may have bearing on the Board's decision in the current appeal.

## STATUS OF CLAIMS

Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40, and 42-45 are pending. All of Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40, and 42-45 are being appealed.

There is only one rejection in the case: all of Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40, and 42-45 stand rejected under 35 U.S.C. §103(a) over Mizutani et al. (*Microbiol. Immunol.*, Vol. 42(8), pp. 549-553, 1998) and Ambion, Inc., TechNotes 8(2) "SUPERase•In: The Right Choice for Protecting Your RNA," hereinafter "Ambion."

## STATUS OF AMENDMENTS

The present application has been the subject of one prior Request for Continued Examination. A Final Office Action was mailed July 3, 2008. The Final Office Action contained a single rejection of all of Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40, and 42-45 under §103(a) over Mizutani et al. and Ambion. No amendments have been filed subsequent to the final rejection.

## SUMMARY OF CLAIMED SUBJECT MATTER

The independent claims on appeal are Claims 1, 10, 18, 26, 33, and 40. There are no "means plus function" or "step plus function" elements in the claims.

The current claimed methods involve heating an RNase inhibitor to protect RNA from enzymatic degradation by RNases. Heating RNase inhibitors to such temperatures was unexpectedly found by the Appellants to increase its RNase-inhibiting activity.

Claim 1 is directed to a method for protecting RNA from enzymatic degradation by RNases. See specification, page 7, lines 6-7 (hereinafter Spec., p. 7, lines 6-7). The method recited in this claim comprises, adding to a first solution containing RNA or to which RNA will

subsequently be added a second solution comprising an amount of an RNase inhibitor protein disposed in a buffer that is devoid of reducing agents, to yield a mixture (Spec., p. 7, lines 7-10). The amount of RNase inhibitor protein in the second solution is sufficient to protect RNA from enzymatic degradation by RNases in the mixture (Spec., p. 7, lines 10-12). The RNase inhibitor protein is derived from rats, human placentas, or recombinant human placental sources (Spec., p. 7, lines 24-25). The mixture is then heated to a temperature no less than about 90°C for a time sufficient to inhibit RNase activity present in the mixture (Spec., p. 7, lines 18-19). The RNA present in the mixture or subsequently added to the mixture is protected from enzymatic degradation by RNases (Spec., p. 7, lines 16-18).

Claim 10 is directed to a method of inactivating RNases in a first solution containing RNA and suspected of containing RNases (Spec., p. 8, lines 6-7). The method recited in this claim comprises adding to the first solution a second solution comprising an RNase inhibitor protein deposited in a buffer that is devoid of reducing agents to yield a mixture (Spec., p. 8, lines 7-10). The RNase inhibitor protein is derived from rats, human placentas, or recombinant human placental sources (Spec., p. 7, lines 24-25). The mixture is then heated to a temperature of at least about 50°C for a time sufficient to inhibit RNase activity present in the mixture (Spec., p. 8, lines 10-11). This results in RNases present in the first solution, if any, being inactivated (Spec., p. 8, lines 11-12).

Claim 18 is directed to a method of storing RNA under conditions that protect the RNA from enzymatic degradation by RNases (Spec., p. 8, lines 14-15). The method recited by this claim comprises adding to a first solution containing isolated RNA or to which isolated RNA will subsequently be added a second solution comprising an RNase inhibitor protein in a buffer devoid of reducing agents to yield a mixture (Spec., p. 8, lines 15-18). The RNase inhibitor protein is derived from rats, human placentas, or recombinant human placental sources (Spec., p. 7, lines 24-25). The mixture is then heated to a temperature of at least about 90°C for a time sufficient to inhibit RNase activity present in the mixture (Spec., p. 7, lines 18-19). The mixture is then cooled (Spec., p. 8, lines 20-21).

Claim 26 is directed to a method of performing RT-PCR and quantitative RT-PCR (Spec., p. 8, lines 22-23). The method recited in this claim comprises first, prior to undergoing thermal cycling, adding to an RT-PCR reaction cocktail containing RNA or to which RNA will subsequently be added an amount of a solution comprising an RNase inhibitor protein in a buffer devoid of reducing agents to yield a mixture (Spec., p. 8, lines 23-27). The RNase inhibitor

protein is derived from rats, human placentas, or recombinant human placental sources (Spec., p. 7, lines 24-25). The amount of the solution added is sufficient to protect any RNA present in the RT-PCR reaction cocktail from enzymatic degradation during a first round of thermocycling (Spec., p. 8, line 27 to p. 9, line 1). Then, if RNA is absent from the mixture, RNA template is added to the mixture (Spec., p. 9, line 2). An RT-PCR reaction is then conducted on the mixture, whereby RNA in the mixture is protected from enzymatic degradation by RNases present in the RT-PCR reaction cocktail and is also protected from enzymatic degradation by RNases (Spec., p. 9, lines 2-6).

Claim 33 is directed to a variation of the method of performing RT-PCR and quantitative RT-PCR recited in Claim 26 (Spec., p. 9, line 7). The method recited in Claim 33 comprises adding a first solution containing an RNase inhibitor protein in a buffer devoid of reducing reagents to an RT-PCR reagent mixture, to yield a second solution (Spec., p. 9, lines 7-8). The RNase inhibitor protein is derived from rats, human placentas, or recombinant human placental sources (Spec., p. 7, lines 24-25). The second solution is then heated to at least about 55°C for a time sufficient to inhibit RNase activity present in the second solution (Spec., p. 8, lines 1-2). RNA is then added to the second solution to yield an RNA mixture (Spec., p. 9, lines 10-11). Lastly, an RT-PCR reaction is conducted on the RNA mixture, whereby the RNA in the RNA mixture is protected from enzymatic degradation by RNases present in the second solution and whereby the RNA in the mixture is further protected from RNases during the RT-PCR reaction (Spec., p. 9, lines 11-14).

Claim 40 is directed to a method of inactivating a prokaryotic or plant RNase (Spec., p. 6, lines 21-24 and p. 11, lines 12-13). The method comprises adding to a first solution suspected of containing a prokaryotic or plant RNase (Spec., p. 6, lines 21-24 and page 11, lines 12-13) a second solution comprising an RNase inhibitor protein in a buffer that is devoid of reducing agents, to yield a mixture (Spec., p. 7, lines 7-10 and p. 8, lines 6-7). The RNase inhibitor protein is derived from rats, human placentas, or recombinant human placental sources (Spec., p. 7, lines 24-25). The mixture is then heated to a temperature of at least about 55°C for a time sufficient to inhibit prokaryotic or plant RNase activity present in the mixture, whereby prokaryotic and plant RNase present in the first solution is inactivated (Spec., p. 8, lines 1-2).

## GROUND OF REJECTION TO BE REVIEWED ON APPEAL

- I. Whether a *prima facie* case of obviousness has not been established because Mizutani et al. and Ambion are silent or teach away from heating RNase inhibitors to increase RNase-inhibiting activity.
- II. Whether a *prima facie* case of obviousness has not been established because there is no technological reason or motivation to combine Mizutani et al. and Ambion.
- III. Whether the rejection is improper because Mizutani et al. and Ambion do not teach heating an RNase inhibitor for a time sufficient to inhibit RNase activity.

## ARGUMENT

### Introduction:

The claimed methods all derive from a discovery that heating RNase inhibitors unexpectedly increases their ability to inhibit RNases. This discovery was unexpected, as the prior art taught that heating RNase inhibitors inactivated the inhibitors and released latent RNase activity from the inhibitors. The current claims recite methods comprising heating a solution containing an RNase inhibitor to various temperatures (90°C for Claims 1 and 18, 50°C for Claim 10, and 55°C for Claims 33 and 40) for a time sufficient to inhibit RNase activity present in the mixture.

The sole rejection in the application is an obviousness rejection over the combination of Mizutani et al. and Ambion. The Office cites Mizutani et al. as teaching heating mixtures containing RNA to 95°C (see page 9, last paragraph of Office Action dated December 10, 2007). The Office cites Ambion as teaching a solution containing an RNase inhibitor protein and heating the solution to 67°C (see page 10, first paragraph of Office Action dated December 10, 2007).

Appellants traverse the rejection on three grounds. Appellants submit that the Office has not established a *prima facie* case of obviousness, first, because the references are silent with respect to, or teach away from, the claimed methods and, second, because there is no technological reason or motivation to combine the teachings of the prior art. Third, Appellants submit that the references in combination fail to teach or suggest all the required elements.

**I. A *prima facie* case has not been established because Mizutani et al. and Ambion are silent or teach away from heating RNase inhibitors to increase RNase-inhibiting activity:**

Appellants submit that the Office has not established a *prima facie* case of obviousness because Mizutani et al. are silent with respect to protecting RNA and Ambion teaches away from heating RNases. Mizutani et al. teach an RT-PCR reaction, which comprises a reverse transcription step in which an RNA solution is heated to 95°C. However, there is no teaching in Mizutani et al. to warrant adding an RNase inhibitor and heating it to the currently claimed temperatures. First, the RT-PCR reaction of Mizutani et al. occurs in the absence of RNase inhibitors. Second, Mizutani et al. are completely silent with respect to RNases or any type of RNase inhibitor. Third, Mizutani et al. are silent with respect to any means of mitigating effects of RNases. On these grounds, the Office is in agreement: “Applicants [*sic*] submission that the Mizutani et al. paper is silent with respect to an RNase inhibitor and [that] they do not use any type of RNase inhibitor, is acknowledged...” (see page 7, first full paragraph of Office Action dated July 3, 2008). Thus, Appellants submit that from the teaching of Mizutani et al., there is no motivation to add an RNase inhibitor to the RNA-containing RT-PCR reaction taught therein. The motivation to add an RNase inhibitor to the RT-PCR reaction taught by Mizutani et al. must therefore derive from Ambion.

Ambion teaches heating solutions containing RNase inhibitors to a temperature of 67°C, as noted by the Office (see page 10, first paragraph of Office Action dated December 10, 2007). The present invention recites heating RNase inhibitors as a means of increasing RNase-inhibition. In stark contrast, Ambion teaches that heating RNase inhibitors increases latent RNase activity. See Figures 2 and 3 of Ambion. The legends of Figures 2 and 3 of Ambion teach that heating the RNase inhibitors to 67°C for 15 minutes releases bound contaminating RNases (legend of Figure 2) and inactivates the inhibitors (legend of Figure 3). Figures 2 and 3 of Ambion show that heating RNase inhibitors to 67°C actually increases RNase activity. The latent RNase activity released by the RNase inhibitors upon heating would destroy any RNA in the solution. Thus, Ambion teaches that heating RNase inhibitors not only decreases ability of RNase inhibitors to inhibit degradation of RNA by RNases but stimulates RNase activity and is therefore counterproductive.

In sum, Mizutani et al. are silent with respect to RNases or any type of RNase inhibitor. Ambion teaches heating RNase inhibitors but teaches that it stimulates latent RNase activity in

the inhibitors. Thus, the combination of references collectively teach against heating RNase inhibitors to maintain their efficacy.

Against Appellants' position that Ambion's teaching that heating releases RNase activity teaches away from combining the RNase of Ambion with heating taught by Mizutani et al., the Office has stated, in relevant part: "[A]t the point of the obvious method at which the temperature is raised to 90°C, the RNase inhibitor is no longer necessary. Thus applicant's traversal of the motivation upon this basis is flawed" (see page 7, second full paragraph of Office Action dated July 3, 2008). In essence, the Office here has argued that the release of latent RNase activity does not detract from establishing a motivation to combine the RNase inhibitor of Ambion with the heating step of Mizutani et al. because there is no need to inhibit RNases in the heating step in Mizutani et al. in the first place. Appellants submit that the Office's position is untenable. If the RNase inhibitor is no longer necessary at the heating step, as the Office contends, then there is no technological motivation to combine the methods. Conversely, the only justification for adding an RNase inhibitor to the heating step is that the RNase-inhibiting activity is beneficial to the RT-PCR method of Mizutani et al., in which case, the release of latent RNase activity upon heating teaches away from the combination. Either position taken by the Office logically yields at least one reason against making the combination. Appellants submit that the Office's response to Appellants' position is untenable and that the Office has failed to establish a motivation to combine the teachings.

## **II. A *prima facie* case of obviousness has not been established because there is no technological reason or motivation to combine Mizutani et al. and Ambion:**

Appellants further submit that there is no technological reason or motivation to combine the two references to yield the invention as claimed because the references do not teach or suggest any benefit to the combination.

The Office has cited Mizutani et al. as heating an RNA solution to 95°C as a step in an RT-PCR reaction (see page 9, final paragraph of Office Action dated December 10, 2007), thereby putatively providing the step of heating Ambion's RNase inhibitor. In general, the objective in an RT-PCR reaction is to obtain amplified DNA from an RNA template. The process occurs, first, by reverse transcribing the RNA template into complementary DNA (cDNA) and, second, by amplifying the cDNA through the polymerase chain reaction (PCR). Because the objective of RT-PCR is to obtain amplified DNA, the stability of the RNA template

after it has been reverse transcribed is inconsequential to the outcome of the method. This applies in the case of Mizutani et al., in which RT-PCR is used to detect the presence of amplified DNA from the RNA genome of hepatitis C virus (HCV) for the purpose of diagnosing HCV infection. Thus, after the RNA has been reverse transcribed into cDNA in the RT-PCR method taught by Mizutani et al., the stability of the original RNA template has no bearing on the outcome of the assay.

In Mizutani et al., the RNA-containing solution is heated to 95°C **only after** the HCV RNA template has been reverse transcribed, when the stability of the RNA is inconsequential to the outcome of the assay. The Office is in agreement with this point. Specifically, the Office has stated, in relevant part, “[A]t the point of the obvious [*i.e.*, Appellants’] method at which the temperature is raised to 90°C, the RNase inhibitor is no longer necessary” (see page 7, second full paragraph of Office Action dated July 3, 2008). Appellants submit that there is no motivation or technical reason to add an RNase inhibitor to the protocol of Mizutani et al. at the point at which the reaction temperature is elevated to 95°C because it would provide no benefit to the assay. On this ground, Appellants submit that the Office has not established a *prima facie* case of obviousness.

Against Appellants’ position that there is no motivation or technical reason to add an RNase inhibitor to the method of Mizutani et al., wherein the reaction temperature is elevated to 95°C, because the RNA would already have been reverse transcribed, the Office has stated that the inhibitor would nevertheless “be present and applicants [*sic*] claims would be obvious” (See page 7, line 2 of Office Action dated July 3, 2008). The Office’s argument, however, assumes as a premise that a motivation to combine the references has already been established, which is exactly the point at issue. Appellants submit that the Office has improperly assumed as given the sought conclusion in rejecting Appellants’ position.

In response to Appellants’ position that there is no technological reason or motivation to combine the two references, the Office has additionally stated:

The motivation for the inclusion of SUPERnasin Ribonuclease inhibitor in the methods of RT-PCR taught by Mizutani et al., is that SUPERnasin inhibits RNases that are known contaminants of RNA preparations. Further SUPERnasin works well in RT-PCR reactions and does not need reducing conditions or reducing agents. (See page 10, second paragraph of Office Action dated December 10, 2007.)



In response, Appellants contend that:

1. Ambion does not teach that RNases are known contaminants of all RNA preparations; and
2. Ambion does not identify RT-PCR as an assay in which RNase contamination is inherently a concern.

Regarding Point 1, Ambion suggests several means by which RNase contamination “might originate” (see line 5 of section entitled “Inhibit More RNases Than Any Other Inhibitor” in Ambion). Ambion teaches RNase contamination only as a possibility and lists several possible sources of the contamination. Ambion does not teach that RNase contamination is known to be present in all RNA preparations; nor does it teach that contamination is inherent in the possible sources listed. It teaches only that in the sources listed, contamination might be present. Thus, Ambion does not suggest that RNases are known contaminants of all RNA preparations.

Regarding Point 2, Ambion teaches that the RNase inhibitor described therein “can be used in any application where RNase contamination is a concern” (see lines 5-6 of section entitled “SUPERase•IN™: The Right Choice for Protecting Your RNA” in Ambion). However, it does not explicitly identify RT-PCR as an assay where RNase contamination is a concern. Ambion does teach that SUPERase•In is compatible for use in RT-PCR because it does not interfere with enzymes used in RT-PCR such as reverse transcriptase or Taq polymerase. However, compatibility in terms of inertness of one substance with another does not constitute a positive motivation to merge the two substances. As reiterated from above, Ambion does not identify RNase contamination as an inherent concern in RT-PCR.

The deficiency in Ambion, *i.e.*, that it does not identify RNase contamination as an inherent concern in RT-PCR, is not rectified by its combination with Mizutani et al. Mizutani et al. teach a protocol for a single-step RT-PCR reaction in the absence of RNase inhibitors, and they are silent with respect to RNases, RNase inhibitors, or any other means of inhibiting RNases. This is *prima facie* evidence that RNases are not known as inherent contaminants in RT-PCR reactions and specifically are not a concern in the RT-PCR method of Mizutani et al.

Because neither of the cited prior art references provides any suggestion that RNases are known contaminants in RT-PCR reactions, Appellants submit that the Office’s response to

Appellants' position that there is no technological reason or motivation to combine the two references is insufficient to establish a *prima facie* case of obviousness.

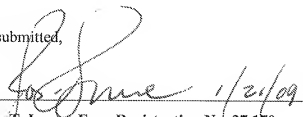
**III. The rejection is improper because Mizutani et al. and Ambion do not teach heating an RNase inhibitor for a time sufficient to inhibit RNase activity:**

Appellants submit that even if the references were to be combined, they fail to teach all the required elements of the claims. The independent claims require heating an RNase for a time sufficient to inhibit RNase activity. The combined references fail to teach heating an RNase for a time sufficient to inhibit RNase activity. Ambion does not teach inhibiting RNase activity with RNase inhibitors at temperatures above 67°C for any amount of time and teach that heating RNase inhibitors at 67°C for 15 minutes increases RNase activity rather than inhibiting it. Mizutani et al. do not teach effects of RNase inhibitors at all. Thus, the combined references fail to teach heating an RNase for a time sufficient to inhibit RNase activity. All the claimed elements are not met. Appellants therefore submit that the combination of references fails to render obvious Appellants' claims.

**CONCLUSION**

In light of the above arguments, the Board is therefore respectfully requested to reverse the only rejection now of record and to allow all of Claims 1, 5, 7-10, 14-18, 22, 24-29, 31-35, 37-40, and 42-45.

Respectfully submitted,



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## CLAIMS APPENDIX

1. (PREVIOUSLY PRESENTED) A method for protecting RNA from enzymatic degradation by RNases, the method comprising:

(a) to a first solution containing RNA or to which RNA will subsequently be added, adding a second solution, the second solution comprising an amount of an RNase inhibitor protein disposed in a buffer that is devoid of reducing agents, to yield a mixture, wherein the amount of RNase inhibitor protein in the second solution is sufficient to protect RNA from enzymatic degradation by RNases, wherein the RNase inhibitor protein is derived from rats, human placentas, or recombinant human placental sources; and then

(b) heating the mixture of step (a) to a temperature no less than about 90°C for a time sufficient to inhibit RNase activity present in the mixture; whereby RNA present in the mixture or subsequently added to the mixture is protected from enzymatic degradation by RNases.

2. - 4. (CANCELED)

5. (PREVIOUSLY PRESENTED) The method of claim 1, wherein in step (a), the RNase inhibitor protein is rat-derived placental RNase inhibitor or human-derived placental RNase inhibitor.

6. (CANCELED).

7. (ORIGINAL) The method of claim 1, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.

8. (ORIGINAL) The method of claim 1, wherein in step (b), the mixture is heated for at least about five (5) minutes.

9. (ORIGINAL) The method of Claim 1, which is a method of protecting RNA from enzymatic degradation by RNase A, RNase B, RNase C, and RNase I.

10. (PREVIOUSLY PRESENTED) A method of inactivating RNases in a first solution containing RNA and suspected of containing RNases, the method comprising:

(a) to the first solution, adding a second solution comprising an RNase inhibitor protein derived from rats, human placentas, or recombinant human placental sources deposited in a buffer that is devoid of reducing agents to yield a mixture; and then

(b) heating the mixture of step (a) to a temperature of at least about 50°C for a time sufficient to inhibit RNase activity present in the mixture; whereby RNases present in the first solution, if any, are inactivated.

11. - 13. (CANCELED)

14. (PREVIOUSLY PRESENTED) The method of claim 10, wherein in step (a), the RNase inhibitor protein is rat-derived placental RNase inhibitor or human-derived placental RNase inhibitor.

15. (ORIGINAL) The method of claim 10, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.

16. (ORIGINAL) The method of claim 10, wherein in step (b), the mixture is heated for at least about five (5) minutes.

17. (ORIGINAL) The method of Claim 10, which is a method of inactivating any RNase A, RNase B, RNase C, and RNase I present in the first solution.

18. (PREVIOUSLY PRESENTED) A method of storing RNA under conditions that protect the RNA from enzymatic degradation by RNases, the method comprising:

(a) to a first solution containing isolated RNA or to which isolated RNA will subsequently be added, adding a second solution comprising an RNase inhibitor protein derived from rats, human placentas, or recombinant human placental sources in a buffer that is devoid of reducing agents, to yield a mixture; and then

(b) heating the mixture of step (a) to a temperature of at least about 90°C for a time sufficient to inhibit RNase activity present in the mixture; and then

(c) cooling the mixture.

19. - 21. (CANCELED)

22. (PREVIOUSLY PRESENTED) The method of claim 18, wherein in step (a), the RNase inhibitor protein is rat-derived placental RNase inhibitor or human-derived placental RNase inhibitor.

23. (CANCELED)

24. (ORIGINAL) The method of claim 18, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.

25. (ORIGINAL) The method of claim 18, wherein in step (b), the mixture is heated for at least about five (5) minutes.

26. (PREVIOUSLY PRESENTED) A method of performing RT-PCR and quantitative RT-PCR, the method comprising:

(a) prior to undergoing thermal cycling, adding to an RT-PCR reaction cocktail containing RNA or to which RNA will subsequently be added, an amount of a solution comprising an RNase inhibitor protein derived from rats, human placentas, or recombinant human placental sources in a buffer that is devoid of reducing agents, to yield a mixture, wherein the amount of the solution added is sufficient to protect any RNA present in the RT-PCR reaction cocktail from enzymatic degradation during a first round of thermocycling; and then

(b) adding RNA template to the mixture of step (a) if RNA is absent, and then conducting an RT-PCR reaction on the mixture of step (a), whereby RNA in the mixture is protected from enzymatic degradation by RNases present in the RT-PCR reaction cocktail and is further protected from enzymatic degradation by RNases.

27. (ORIGINAL) The method of claim 26, wherein after step (a) and prior to step (b), the mixture is heated to a temperature no less than about 55°C.

28. (ORIGINAL) The method of claim 26, wherein after step (a) and prior to step (b), the mixture is heated to a temperature greater than 65°C.

29. (ORIGINAL) The method of claim 26, wherein after step (a) and prior to step (b), the mixture is heated to a temperature no less than about 70°C.

30. (CANCELED)

31. (PREVIOUSLY PRESENTED) The method of claim 26, wherein in step (a), the RNase inhibitor protein is rat-derived placental RNase inhibitor or human-derived placental RNase inhibitor.

32. (ORIGINAL) The method of claim 26, wherein in step (a) the RT-PCR reaction cocktail does not contain RNA; and after step (a) and prior to step (b), the mixture is heated to at least about 90°C.

33. (PREVIOUSLY PRESENTED) A method of performing RT-PCR and quantitative RT-PCR, the method comprising:

(a) to an RT-PCR reagent mixture, adding a first solution containing an RNase inhibitor protein derived from rats, human placentas, or recombinant human placental sources in a buffer, the buffer being devoid of reducing agents, to yield a second solution; and

(b) heating the second solution to at least about 55°C for a time sufficient to inhibit RNase activity present in the second solution; and then

(c) adding RNA to the second solution to yield an RNA mixture; and then

(d) conducting an RT-PCR reaction on the RNA mixture of step (c); whereby the RNA in the RNA mixture is protected from enzymatic degradation by RNases present in the second solution and whereby the RNA in the mixture is further protected from RNases during the RT-PCR reaction.

34. (ORIGINAL) The method of claim 33, wherein in step (b), the second solution is heated to a temperature no less than about 70°C.

35. (ORIGINAL) The method of claim 33, wherein in step (b), the second solution is heated to a temperature no less than about 90°C.

36. (CANCELED)

37. (PREVIOUSLY PRESENTED) The method of claim 33, wherein in step (a), the RNase inhibitor protein is rat-derived placental RNase inhibitor or human-derived placental RNase inhibitor.

38. (ORIGINAL) The method of claim 33, wherein in step (b), the mixture is heated for at least about twenty (20) seconds.

39. (ORIGINAL) The method of claim 33, wherein in step (b), the mixture is heated for at least about five (5) minutes.

40. (PREVIOUSLY PRESENTED) A method of inactivating a prokaryotic or plant RNase comprising:

(a) to a first solution suspected of containing a prokaryotic or plant RNase, adding a second solution comprising an RNase inhibitor protein derived from rats, human placentas, or recombinant human placental sources in a buffer that is devoid of reducing agents, to yield a mixture; and then

(b) heating the mixture of step (a) to a temperature of at least about 55°C for a time sufficient to inhibit prokaryotic or plant RNase activity present in the mixture, whereby prokaryotic and plant RNase present in the first solution is inactivated.

41. (CANCELED)

42. (PREVIOUSLY PRESENTED) The method of claim 40, wherein in step (a), the RNase inhibitor protein is rat-derived placental RNase inhibitor or human-derived placental RNase inhibitor.

43. (ORIGINAL) The method of claim 40, wherein in step (b), the mixture is heated for at least about twenty (20) second.

44. (ORIGINAL) The method of claim 40, wherein in step (b), the mixture is heated for at least about five (5) minutes.

45. (ORIGINAL) The method of claim 40, wherein in step (a), the first solution is suspected of containing E. coli RNase; and in step (b), the mixture is heated for a time sufficient to inhibit E. coli RNase activity present in the mixture.

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## EVIDENCE APPENDIX

None.

## RELATED PROCEEDINGS APPENDIX

An appeal is pending in U.S. Application No. 10/403,395.